

## AMENDMENTS TO THE SPECIFICATION

Please make the following amendments (underlining for added matter and ~~strikethroughs~~ or ~~[[brackets]]~~ for deleted matter) to the specification:

[0034] *Figure 20* shows a sequence of images showing the progress of mixing of yellow dyed-Columbo™ yogurt and blue dye in a device with straight-sided deflectable portions.

[0052] Figures 2A and 2B depict an exemplary embodiment of a device **10** for fluid mixing and gas exchange in accordance with the invention. Figure 2A is a perspective view showing a chamber **12** which may also be referred to as a “well” or “growth well” herein, located within housing **14**. ~~The darker blue area within the lighter blue housing represents the chamber, which may also be referred to as a “well” or “growth well” herein.~~ The device may be placed on a substrate, e.g., a planar substrate such as a glass slide. Figure 2B shows an exploded view of the device, in which the interior of the chamber is visible. While the chamber depicted in Figure 2 is rectangular in cross section in the xy, xz, and yz planes, it will be appreciated that any of a large number of different chamber geometries may be used. For purposes of description herein, and in reference to a particular embodiment of the invention that has been constructed and tested (see below), it will be assumed that the chamber is rectangular in cross section as shown in Figure 2. However, it is to be understood that the chamber may assume other geometries, e.g., cubical, cylindrical, conical. Typically the length of the chamber in the z direction will be less than its length in the x and y directions.

[0054] The top wall of the chamber in Figure 2 comprises 7 deflectable portions **16**, ~~indicated in black~~ in both Figures 2A and 2B. The deflectable portions extend in the -y direction through the width of the chamber in the y dimension. Figures 2A and 2B also depict a hollow space **18**, ~~indicated in red~~, located adjacent to and in contact with each deflectable portion. In Figures 2A and 2B, hollow spaces **18** extend in the -y direction through the width of the chamber in the y dimension. However, in general, a hollow space **18** need not extend through the entire width of a chamber and need not contact the entire corresponding deflectable portion. In certain embodiments of the invention hollow spaces **18** form an integral part of the wall of the

chamber that comprises the deflectable portions. Thus the deflectable portions of the chamber wall also form part or all of the wall of the hollow space where it adjoins the chamber. Thus the hollow space is in contact with the deflectable portion since the deflectable portion provides part or all of the wall of the hollow space. In other embodiments of the invention hollow spaces **18** are external to the wall that comprises the deflectable portions. It will be appreciated that in the latter case the hollow spaces should be fabricated in a material that is permeable to the gas of interest, so that the gas may diffuse from the hollow spaces into the deflectable portions of the wall. The deflectable portions themselves may be individual, i.e., physically separated from each other by a intervening region, which may be made from a different material. Alternately, the deflectable portions may be part of a single layer or membrane, which forms the wall of the chamber. Individual portions of the layer or membrane are selectively deflectable, e.g., by virtue of their contact with individual hollow spaces that can be selectively pressurized as described below.

**[0058]** Figure 3 shows a schematic cross-sectional view of a deflectable portion **16** ~~shown as the blue area between solid black lines~~ and a chamber **12** ~~shown in green~~. Hollow space **18**, ~~shown as the dark blue area between solid black lines~~, overlies the deflectable portion. The upper portion of Figure 3 shows the unactuated (low pressure) state in which the portion is not deflected. The lower portion of the figure shows the actuated (high pressure) state in which the portion is deflected into the chamber except at the perimeter, which is constrained. In an actual device, the chamber would extend in the left and right directions, and the top would comprise additional deflectable portion(s). Figure 3 shows an embodiment of the device that is constructed in layers in which adjacent deflectable portions are physically joined and adjacent hollow spaces are located within a continuous layer of material. While pressurization of the cavity is a preferred means of causing deflection, other deflection methods can also be used.

**[0061]** Figures 4A – 4C show an enlarged view of the chamber and an actuation sequence of the deflectable portions to achieve peristaltic action. Figure 4A shows a cross-sectional view of the chamber **12** (growth well) ~~shown in green~~ and hollow spaces **18** ~~shown in dark blue~~ adjacent to deflectable portions **16** of the top wall, ~~shown in black~~, in the unactuated state. Figure 4B shows simultaneous actuation of the leftmost two deflectable portions, which

may be achieved by pressuring the corresponding hollow spaces directly above. Deflection of the portions into the chamber causes ~~displacement~~ displacement of the contents of the chamber below the deflected regions, forcing the contents to move elsewhere in the chamber. The increased pressure in the chamber results in upward displacement of the undeflected portions, as shown. Figure 4C depicts subsequent de-actuation of the leftmost portion (e.g., by releasing the pressure in the hollow cavity above), continued deflection of the second portion, and actuation of the third portion from the left. Deflection of the third portion from the left pushes the chamber contents further in the rightward direction. In addition, upon release of the leftmost portion, chamber contents move backward into the region that was previously occupied by the deflected region.

**[0062]** The pattern shown in Figure 4 proceeds to the right and wraps around to the left when it reaches the right edge. Thus according to this deflection sequence 2 portions are deflected at any given time. Such a sequence may be represented as follows, with a “0” representing an undeflected portion and a “1” representing a deflected portion: 0000000 (starting state); 1100000; 0110000; 0011000; 0001100; 0000110; 0000011; 1000001; 1100000 (return to first actuated state). Figure 5 shows a top view of the deflection pattern in the unactuated state (Figure 5A) and the first two actuated states. ~~Unactuated portions are indicated in teal, while deflected portions are indicated in dark blue.~~ In Figure 5B the two leftmost portions are deflected, and fluid is ~~displaced~~ displaced to the right and upwards, i.e., the unactuated portions are displaced upwards into the overlying hollow cavities. In Figure 5C the second portion remains deflected, the first portion is released, and the third portion is deflected. Fluid is pushed to the right by the third portion and around the second portion to push up the first portion.

**[0063]** Figure 6 shows the approximate flow of fluid in the chamber for straight deflectable portions deflected according to the sequence presented above. ~~As in Figure 5, undeflected portions are shown in teal while deflected portions are shown in dark blue.~~ Large arrows indicate the actuation sequence. Small arrows in each chamber indicate the direction of fluid flow. The overall flow pattern is shown in the center of the figure ~~using red arrows~~. In general, the action of the device achieves an overall circular pattern of fluid flow rather than a unidirectional flow. Circulation of the fluid in this manner mixes the contents of the chamber. As the contents of the chamber move, they come into contact with the O<sub>2</sub>-rich region near the

deflectable portions, thereby increasing the rate of O<sub>2</sub> transfer. In general, a wide variety of different deflection sequences may be used. For example, a single portion may be deflected at any given time. Other suitable sequences include 1000001, 0100010, 0010100, 0001000, 0010100, 0100010, 1000001 (in which deflectable portions are sequentially pressurized beginning at either end of the wall and the deflection pattern goes back and forth in opposite directions); 1100000, 01100000, 0011000, 0001100, 0000110, 0000011, 0000110, 0001100, 0011000, 0110000, 1100000 (deflectable portions are deflected in groups of two but the pattern goes back and forth from opposite ends of the chamber instead of wrapping around), etc. It will be appreciated that the sequences will vary depending upon the number of deflectable portions.

**[0067]** Figure 7B shows an alternate design in which the 7 hollow cavities **18** comprise 3 groups (the first group comprises the leftmost two cavities, the second group comprises the three cavities in the middle, and the third group comprises the rightmost two cavities~~shown in blue, orange, and light green~~), each of which is controllable independent of the other 2 groups. One of the hollow cavities in each group (the leftmost cavity in each group) is connected to a valve **20**, while the remaining cavities are not connected to a valve. A small hollow connection **22** exists between the members of each group. The leftmost cavity in each group is first to pressurize upon actuation of the valve to which it is connected. The cavity immediately to the right of each cavity connected to a valve pressurizes next, etc. The small size of the connection causes a delay so that the members of each group will pressurize in sequence rather than together.

**[0069]** Figure 8 shows a schematic of a top view of an embodiment in which the width of the deflectable portions is variable and in which the portions do not all have identical shapes. Adjacent portions contain complementary convex and concave regions. Except for the middle portion, one side of each portion has both a concave and a convex region, which “fits” into complementary convex and concave regions on the adjacent portion. The other sides are straight. The middle portion contains two convex and two concave portions, which correspond to complementary concave and convex regions of the adjacent portions. Large arrows on Figure 8 show the actuation sequence while small arrows on each chamber show the approximate direction of fluid flow. It will be appreciated that the direction of flow exhibits less uniformity than in the embodiment described above. The central image shows the overall direction of fluid flow (~~red arrows~~). The action of the device results in a number of approximately circular flow

patterns. Results (see below) indicate that mixing takes place considerably faster when cavities with variable widths are used.

**[0099]** Figure 18 shows a larger view of the device in which the actuation interface ports and injection ports are visible. ~~Two distinct dyes~~ Yellow and blue dye in water were injected into at opposite ends of the device via the injection ports. The device was actuated at 100 Hz, ~10 psi. Figure 19 presents a sequence of images showing the progress of mixing. The upper left image shows mixing in an unactuated device at  $t > 1$  hour after injection of the dyed water into the chamber. It is evident that very little mixing has occurred. The image in the upper right shows the chamber 5 seconds after actuation. ~~It is evident that the water containing the yellow dye has been pushed forward and that backwards movement of the blue dyed water is beginning.~~ The lower images show mixing after 20 seconds (left) and 45 seconds (right) of actuation. By 45 seconds the contents of the chamber are almost a uniform mixture of the two dyes shade of green.

**[00100]** Figure 20 shows a similar sequence for the mixing of ~~yellow dyed~~ Columbo™ yogurt and blue dye in water (viscosity 1 cp). Dual circulation flow is evident, and mixing takes place in approximately 18 minutes, demonstrating the ability of the device to mix even extremely viscous fluids. Mixing of diluted corn syrup (estimated viscosity of 1500cp) has also been demonstrated (not shown).

**[00101]** Figure 21 shows mixing achieved by a device similar to that described above except using cavities (tubes) with nonuniform widths (as shown in the middle mask in Figure 14). ~~Dye~~ Blue dye in water containing 600 g/L glucose ( $1 < \text{viscosity} < 50$  cp) was injected into an access port at one end of the chamber. The deflection rate was 100 Hz, and the pressure was 8 psi. Local circulation flows can be seen. As is evident, mixing is achieved considerably more rapidly using tubes with nonuniform widths than with the straight-sided tubes.

**[00103]** Figure 23 is another sequence showing mixing achieved by a device with cavities (tubes) with nonuniform widths as shown in the middle mask in Figure 14. ~~Dye~~ Blue dye in water or in water containing light corn syrup (1500 cp) was injected into an access port at one end of the chamber. As indicated, the upper left image shows deflection at 10 Hz, 8 psi. The

lower sequence shows mixing at a deflection rate of 100 Hz, and the pressure was 8 psi. The circulation flows are evident, as shown in the diagram at the center of the page.